AF cont.

ATCCCAATTGCCGCC**ATGGTTGATTTGGAGAGCGAAGTG**-3' (24) (SEQ ID NO:38) and the antisense oligo overlapping the stop codon, oligo #20. Only the bases listed in bold type in oligos 20 and 24 match the Slo4 DNA sequence. In oligo 20, the additional bases at the 5' end add an XbaI restriction site to assist subcloning. In oligo 24 the additional bases at the 5' end add a MunI site for subcloning and a Kozak consensus sequence to boost translation initiation at the Slo4 methionine codon. Only the areas given in bold type are used for the amplification of Slo4; the other bases need not be present to obtain amplification.--

Please cancel the present informal "SEQUENCE LISTING", pages 75-77, and insert therefor the accompanying paper copy of the Sequence Listing, page numbers 1 to 30, at the end of the application. Cancel the page numbers of the Claims and Abstract and renumber as pages 75 through 84.

REMARKS

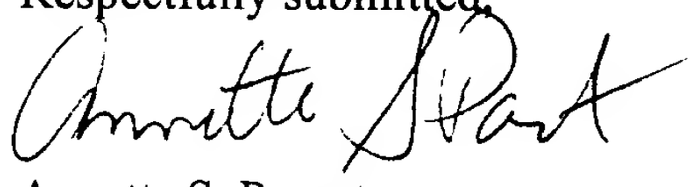
In accordance with 37 C.F.R. §§1.821 to 1.825, Applicants request entry of this amendment. This amendment is accompanied by a floppy disk containing SEQ ID NOS:1-31, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification by the current Amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 26 of page 8 has been amended as follows:

Figure 1. Amino acid alignment of the complete human Slo2 amino acid sequence (SEQ ID NO:2) to the amino acids sequences of rat SLACK (SEQ ID NO:32) and the partial human cDNA KIAA1422 (SEQ ID NO:33). Identical residues are shaded and amino acid numbers are given at the left margin. Human Slo2 and rat SLACK are 93% identical and are probably orthologous genes. The pattern of divergence between human Slo2 and rat SLACK is not typical for orthologous potassium channels. KIAA1422 is a partial cDNA from the hSlo2 gene, but differs in several key ways. First, it has an alternative amino terminus that is highly divergent from those of human Slo2 and rat SLACK. It is also clearly truncated on the 3' end. The human Slo2 DNA and amino acid sequences can not be readily predicted from KIAA1422 or rat SLACK.

Paragraph beginning at line 3 of page 9 has been amended as follows:

Figure 2. Amino acid alignment of human Slo2 (SEQ ID NO:2) and human Slo4 (SEQ ID NO:4). Identical residues are shaded and amino acid numbers are given at the left margin. Six predicted transmembrane domains are underlined with solid lines. The potassium channel "signature sequence" or pore loop is underlined with a dotted line. A dashed line indicates a tail region that has been implicated in Slo channel calcium and chloride gating (Yuan *et al., Nat. Neurosci.* 3:771-779 (2000); Wei *et al., Neuron*, 13:671-681 (1994)). The two amino acid sequences are approximately 70% identical.

Paragraph beginning at line 24 of page 56 has been amended as follows:

Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly Gly ~~gly~~ sequences of between about 5 and 200 amino acids (SEQ ID NO:34). Such flexible linkers are known to persons of skill in the art. For example, poly(ethelyne glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

Paragraph beginning at line 9 of page 68 has been amended as follows:

A clone containing a 5' incomplete (but otherwise full-length) human Slo2 sequence was constructed using overlap extension PCR and 3 Slo2 fragments amplified from human hippocampus cDNA. A 5' fragment of approximately 1.3 Kb was amplified using the sense oligo 5'-CACCTTCAAGGAGCGGCTCAAGCTG-3' (9) (SEQ ID NO:13) and the antisense oligo 5'-GACGTGTGCACCAGCAGGGTGATGAG-3' (10) SEQ ID NO:14). The middle of the Slo2 sequence was amplified as a 1.55 Kb fragment with the oligos (sense) 5'-GTTTCACGTCAAGTTTGCTGACCACG-3' (11) (SEQ ID NO:15) and (antisense) 5'-CCGTACGTGCGGATCCACAGGTCG-3' (12) (SEQ ID NO:16). The 3' end of the Slo2 coding sequence was amplified with the sense oligo 5'-CGTGAAGGACTACATGATCACCATC-3' (13) (SEQ ID NO:17) and the antisense oligo 5'-CAGGGTCTAGA**TTAGAGCTGTGTCTCGTCGCGAGTCTC**-3' (14) (SEQ ID NO:35) ~~(SEQ ID NO:18)~~ to produce a fragment of 800 bp. The latter oligo includes the predicted Slo2 stop codon and 3' end of Slo2 coding (in bold), plus an XbaI site for subcloning on the 5' end. It should be noted that only the bold sequence corresponding to Slo2 is used to amplify Slo2. These fragments were assembled into a single fragment using 2 rounds of standard overlap extension PCR. First, the 5' and middle fragments were mixed and amplified with oligos 9 and 12 to produce a fragment of approximately 2.8 Kb. Similarly, the middle and 3' end fragments were mixed and

amplified with oligos 11 and 14 to produce a fragment of approximately 2.3 Kb. These two larger fragments were then mixed and amplified with oligos 9 and 14 to produce a fragment of 3.5 Kb containing all known human Slo2 sequence. This fragment was cloned into a plasmid vector and multiple clones were sequenced to determine a final human Slo2 sequence for this region. The conditions used to amplify the coding region of Slo2 were similar to those described above for the Slo2 RACE reactions. One notable exception is that longer extension times (4 minutes) were allowed during the overlap reactions because of the large size of the desired fragments.

Paragraph beginning at line 11 of page 69 has been amended as follows:

The 5' end of Slo2 was amplified from human brain cDNA using an overlap extension PCR screen. A fragment containing the start codon and first 200 bp of Slo2 was amplified using the sense oligo 5'-CCACC**ATGGCGCGGGCCAAGCT**-3' (15) (SEQ ID NO:36) ~~(SEQ ID NO:19)~~ and the antisense oligo 5'-GAGACAGGGAGGAGTCCAGGCTGAA-3' (16) (SEQ ID NO:20). Only the bold bases in 15 match Slo2 and are used for amplification of Slo2; the 5' bases add a Kozak consensus sequence and are included only for expression vector construction. A second fragment of approximately 400 bp that overlapped the first fragment and a unique Hind III restriction site in the 3.5 Kb Slo2 clone was amplified using the oligos 5'-CGTGGGCCAGAGGCTTCCTGTAGAA-3' (17) (SEQ ID NO:21) and 5'-GCTCCCAGATGTTGCCTTTGTAGCTG-3' (18) (SEQ ID NO:22). These two fragments were mixed and amplified with oligos 15 and 18 to produce an approximately 550 bp fragment containing both the initiator methionine of Slo2 and the unique Slo2 Hind III site. This fragment was cloned into a standard plasmid and 3 clones were sequenced. Each clone was identical to the consensus human Slo2 derived from our cDNA and genomic information. A full length Slo2 coding region was then assembled by joining the 5' end fragment and 3.5 Kb Slo2 fragment at their common Hind III restriction site by standard DNA cloning methods.

Paragraph beginning at line 20 of page 71 has been amended as follows:

Partial human Slo4 sequences were originally identified with TBLASTN searches of 3 databases with the rat SLACK sequence and partial human Slo2 sequences: A proprietary database, the public EST database at NCBI, and the public Genome Survey Sequence Database at NCBI. The proprietary clone 5035170 contained a short stretch of Slo4 coding sequence with amino acid 60% identity to rat SLACK amino acids 646-730. The entire clone had an insert of less than 700 bp. It was sequenced in its entirety and determined that most of the insert probably represented intronic sequence. The two Genome survey sequences (GSS), AQ701228 and AQ892600 contained homology Rat SLACK just 5' and 3' to the proprietary clone, respectively. Finally, a public EST clone (AI791929) was identified that had homology to the 3' end of the RAT SLACK coding sequence and appeared to contain a stop codon for the Slo4 open reading frame. These non-overlapping sequences were confirmed to have come from the same gene by amplifying an approximately 1.5 Kb fragment with a sense oligo based on the 5' most sequence (AQ701228), 5'-GGCGTCTGCTTGATTGGTGTTAGGA-3' (19) (SEQ ID NO:23), and an antisense oligo overlapping the stop codon in the EST sequence, 5'-TTTATCTAGA**ATCAAAGTTGAGTTTCCTCCCGAG**-3' (20) (SEQ ID NO:37) ~~(SEQ ID NO:24)~~. This amplified clone contained sequence identical to all four of the clones identified by BLAST searches. It also contained a high degree of homology to human Slo2 and rat SLACK across its entire length.

Paragraph beginning at line 29 of page 72 has been amended as follows:

The entire Slo4 coding sequence was amplified in a single fragment using a sense oligo overlapping the initiator methionine codon, 5'-ATCCCAATTGCCGCC**ATGGTTGATTTGGAGAGCGAAGTG**-3' (24) (SEQ ID NO:38) ~~(SEQ ID NO:28)~~ and the antisense oligo overlapping the stop codon, oligo #20. Only the bases listed in bold type in oligos 20 and 24 match the Slo4 DNA sequence. In

oligo 20, the additional bases at the 5' end add an XbaI restriction site to assist subcloning. In oligo 24 the additional bases at the 5' end add a MunI site for subcloning and a Kozak consensus sequence to boost translation initiation at the Slo4 methionine codon. Only the areas given in bold type are used for the amplification of Slo4; the other bases need not be present to obtain amplification.